REMARKS

Review and reconsideration of the Office Action of January 03, 2002, is respectfully requested in view of the above amendments and the following remarks.

Claims 1-22 were previously canceled without prejudice.

Claims 23-51 are pending.

Claims 35-51, non-elected following a restriction requirement, are withdrawn from consideration.

Claims 23-34, drawn to bacteria useful as a vehicle for gene transport and gene transfer in eukaryotic cells, and elected in response to a restriction requirement, are rejected.

No claim has been allowed.

The Restriction Requirement and Election

The election requirement was made FINAL. Claims 35-51 are withdrawn from consideration and are being retained in the case pending a decision to file a divisional application.

Claim Rejections - 35 USC §112 first paragraph

Claims 23-34 stand rejected under 35 U.S.C §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and/or use the invention. Further, in the opinion of the Examiner the specification has failed to provide any guidance, direction, or working examples that demonstrate *in vivo* somatic cell gene transfer using the claimed bacteria. As the state of the art is unpredictable with respect to the use of bacteria as vehicles for *in vivo* somatic cell gene transfer, the skilled artisan cannot rely on the state of the art for teachings that demonstrate the use of bacteria for such purpose. The

examiner concluded that it would have required undue experimentation to use the claimed bacteria for *in vivo* somatic cell gene transfer.

Applicants respectfully traverse.

In United States v. Teletronics, Inc., 857 F.2d 778, 8 U.S.P.Q. 2d 1217, 1223 (CAFC 1988) it was stated that

> [T]the test of enablement is whether a person reasonably skilled in the art could make or use the invention from the disclosure in the patent coupled with information known in the art without undue experimentation.

In the case *In re* Wands, 858 F.2d 731, 737, 8 U.S.P.Q 2d 1400, 1404 (CAFC 1988) the court held that

> the test is not merely quantitative, since a considerable amount of experimentations is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction to which the experimentation should proceed.

Because the specification of a patent is addressed to the person skilled in the art, it is not necessary to explain every detail (DeGeorge v. Bernier, 768 F.2d 1318, 1323-24, 226 U.S.P.Q. 758, 762-63 (CAFC 1985). Every patent application and prior art reference relies to some extent upon knowledge of persons skilled in the art to complement what is disclosed. (*In re* Bode, 550 F.2d 656, 660, 193 U.S.P.Q. 12, 16 (CCPA 1977)). In the case *In re* Certan Limited-Charge Cell Culture Microcarriers, 221 U.S.P.Q. 1165, 1174 (US ITC 1983) it was corroborated that

> [T]the fact that experimentation may be complex does not necessarily make it undue, if the art (in which the

invention is situated) typically engages in such (complex) experimentation.

In further decisions it was emphasized that a patent's disclosure is in compliance with 35 U.S.C §112 if it defines the desired functional relationship, even if some experimentation is required to reproduce the invention. Some experimentation to select operating parameters is also permissible (Syntex, Inc. v. Paragon Optical, Inc., 7 U.S.P.Q. 2d 1001, 1035 (D. Ariz. 1987); *In re* Angstadt, 537 F.2d 498, 502, 190 U.S.P.Q. 214, 218 (CCPA 1976).

The Examiner is correct that biotechnology and gene transfer belong to the so-called unpredictable arts, thus sufficient disclosure must be provided. On the other hand, there was high level of skill in the art at the time when the application was filed, and all of the methods needed to practice the invention were well known. Therefore, the application provides sufficient guidance to the skilled artisan to reproduce the claimed invention.

In the following the Examiner's concerns, which were expressed in the latest Office Action, are addressed in detail. The Examiner is referred to the pending patent application as originally filed and prior art documents cited therein, of which we submit copies for the examiner's convenience.

Efficient *in vivo* gene transfer using intracellular bacteria has four main requirements, namely

(i) the gene transfer must be effective *in vitro*,

(ii) an appropriate host cell line must be chosen,

(iii) the release of the bacteria into the cytosol has to be predictable and must not be accidental, and

(iv) the bacteria have to be able to cause infections *in vivo*.

Referring to (i), in the patent application, gene transfer using *Listeria monocytogenes* was as efficient as about 4 % (see Table 1, page 25 of the description as originally filed). In the meantime the inventors were able to transfer *Listeria monocytogenes* with a transfection efficiency of more than 20 %, wherein only minor chances were made to the protocol disclosed in the patent application. A working protocol and the results of a FACS analysis, which show a transfection rate of more than 20 %, are enclosed for the examiner's convenience. These ongoing studies clearly demonstrate that gene transfer using bacteria was possible at the priority date of the patent application and led to reproducible and reliable results. Therefore, a person skilled in the art did not face undue experimentation.

It is further referred to the above-cited case *In re* Wand, where according to the PTO Board, only 4 out of 143 hybridomas, or 2.8 % were successful. In its decision, the Court emphasized that even if only a success rate of 2.8 % were to be accepted, that would not necessarily mean that the experimentation was undue.

It is stressed that after the priority date of the patent application additional protocols were published, which show a high efficiency of gene transfer using bacteria, just to mention Grillot-Courvalin et al., 1998, Nat. Biotechn., 16:862-866, where 16 % efficiency was reached using modified *E. coli* strains, and Grillot-Courvalin, 2002, Cell. Microbiol., 4:177-186, who reveal transfection rates as high as 12 % using *Listeria monocytogenes*.

Further, Applicants provide for the information of the Examiner a recently discovered reference Duechler et al, J. Gen. Med. 2002, 282-291, published after the priority date of the present application, teaching that it is in fact possible *in vivo* to carry out somatic gene transfer via L. monocytogenes or the other bacteria mentioned in the present patent application, for example in the milk lactating cells of sheep. Further, referring to literature reference number 7, it is indicated that the retrograde transport of virus in the natural target cells with Listeria is known. This further evidences that the person of ordinary skill in this art, at the time the application was filed, was in possession of the knowledge that bacteria such as *Listeria monocytogenes* could be used for the purposes described in detail in the present application.

Referring to (ii), relying on the guidance given in the patent application and to what was known in the prior art, the skilled artisan was able to conduct efficient gene transfer using the claimed bacteria. Besides suitable bacterial strains and plasmids, which are both described in the application, it is important to chose a suitable target cell line. The skilled person was able to select such cell line since 1996, when the first cellular receptor for *Listeria monocytogenes* was published (Mengaud et al., 1996, Cell, 84:923-32). It was disclosed that the cellular protein E-Cadherin is responsible for binding of *Listeria monocytogenes* to eucaryotic cells. From this initial disclosure one can conclude that cells which express E-Cadherin are particular susceptible for transfection with *Listeria monocytogenes*. In the meantime other receptor molecules for

Listeria monocytogenes have been discovered, such as gC1qR and Met (Cossart, Trends Microbiol., 9:105-107). A similar situation is found *in vivo*, and it was known as early as 1972 that *Listeria monocytogenes* infects epithelial cells with high preference (Racz et al., Lab. Investig., 1972, 26:694-700). As demonstrated by Jensen et al., Infect. Immun., 66:3758-3766 epithelial cells can be infected with high efficiency *in vivo*, because these highly specialized cells are used by *Listeria monocytogenes* to enter the host's organism.

(iii) The Examiner asserted that the use of bacteria for gene transfer *in vivo* is unpredictable because the bacteria must first enter the cell and then escape from the vacuole to the cytosol. The movement from the vacuole to the cytosol is considered to be also unpredictable because in many instances the bacteria are lysed by the host cell's defense system and any plasmids carried by the bacteria are degraded preventing expression of heterologous nucleotide sequences. The Examiner cited Grillot-Courvalin et al., 1998, Nature Biotechnology 16:862-866, which was published after the priority date of the present application. The authors reported that *E. coli* carrying a nucleotide sequence encoding GFP was only able to transform 0.3-1.0 % of a transfected macrophages cell line.

The Examiner's argument is well taken; however, Applicants respectfully traverse and the Examiner's attention is drawn to the fact that macrophages and dendritic cells are not the primary target of the claimed bacteria. Both cells are part of the immune system and are known as cells which phagocyte most efficiently (MHC II cells). Such cells are likely to have adopted special properties to destroy DNA derived from microorganism entering the

human or animal body. Such mechanisms may explain why gene transfer in macrophages is less efficient than in other cell types, although it is emphasized that *Listeria monocytogenes* is able to proliferate in macrophages and dendritic cells. In contrast to this, *Listeria monocytogenes* itself forces - as part of its "natural life style" - its entry into cells, which are naturally non-phagocytotic, by inducing a pathway which is called endocytosis. For this purpose the interaction between the cellular receptor E-Cadherin and the bacterial ligand internalin A plays an important roll. Target cells of *Listeria monocytogenes* do not need protection mechanisms like macrophages or dendritic cells, thus efficient gene transfer is observed.

As stated above, the Examiner is correct that biotechnology and gene transfer in general belong to the so-called unpredictable arts. However, applicants traverse the Examiner's opinion that the escape of the claimed bacteria into the cytosol is itself unpredictable because most of the bacteria are lysed and therefore destroyed in endosomes. Technically speaking, exactly the opposite is true. Indeed, intracellular bacteria, such as *Listeria monocytogenes* trigger and force their uptake in endosomes of the respective cells. The process of specific uptake itself is called induced endocytosis. After that, said bacteria escape quickly and efficiently from the endosomes with the help of suitable enzymes like e.g. listeriolysine and phospholipases B and C, and proliferate efficiently in the cytoplasm of the respective cell. Because of these capabilities, intracellular bacteria are most suitable for gene transfer *in vivo* as reflected in the Applicants' choice of bacteria.

Applicants affirm the Examiner's point of view that most pathogens of *Listeria monocytogenes*, which infect a natural host

in vivo, are inactivated and eliminated by the host's immune system. However, it is emphasized that it is well known in literature that a sufficient amount of these bacteria is able to survive and to efficiently infect liver cells, lactating cells and cells of the central nerve system *in vivo* (Cheers et al., 1978, Infect. Immun., 19:763-770). Hence, intracellular bacteria are particularly suitable for *in vivo* gene therapy, as they comprise the natural capability to withstand the pressure of the host's immune system.

Turning now to (iv), it is pointed out that *Listeria monocytogenes* are able to cause infections in humans. Additionally, the state of the art provides animal models, which show clearly that human pathogenic bacteria are able to cause infections *in vivo*. At the priority date of the present application, state of the art literature provided the skilled artisan with knowledge about methods for infecting mice with *Listeria monocytogenes* either via oral uptake or injection (Audurier et al., Ann. Microbiol., 1980, 131B:47-57; Racz et al., 1972, Lab. Investig., 26:694-700:; Marco et al., 1997, Microb. Pathog., 23:255-263; Schleich, 1993, Clin. Invest. Med., 16:219-225). Further, a method for inoculation of bacteria into lactating cells (glands) was disclosed by Bryner et al., 1989, in: Acta Microbiol. Hung., 36:137-140.

Due to that knowledge in the state of the art at the priority date, the skilled artisan was able to carry out *in vivo* gene transfer with any undue burden and, additionally, when using the claimed bacteria, he had a reasonable expectation that *in vivo* gene transfer would be successful.

Next, the Examiner's statement concerning the folding of proteins is addressed. It was said that Pascual et al., 1997, Behring Inst. 98:143-152 reported on page 143-145 that the folding of antigens in the bacterial cytoplasm can affect humoral immunity to discontinuous epitopes such that misfolded antigens expressed by bacterial vectors would be expected to induce humoral immunity only against continuous epitopes and irrelevant discontinuous epitopes; such is a significant limitation of bacterial vaccine vectors. The Examiner is correct that such misfolded proteinaceous antigens affect immunogenicity. However, it is emphasized that such misfolding does not play any role for the inventive technology described in the pending application. In contrast to Pascal et al. the invention uses bacterial vectors to transfer DNA only. Transcription of the DNA to mRNA and the consecutive translation of mRNA into the desired protein take place merely under eucaryotic control. Therefore, the folding of the protein is carried out in the cellular cytoplasm and at the eucaryotic endoplasmatic reticulum so that a correct folding of the protein is ensured at any time.

The Examiner will note that the skilled artisan received sufficient information from the disclosure of the application in order to allow him to carry out *in vivo* gene transfer efficiently and successfully when combining the disclosure of the application with the knowledge of the state of the art.

For the Examiner's convenience the most important steps for *in vivo* gene transfer are briefly summarized. For efficient gene transfer it is primarily necessary for the skilled artisan to have suitable bacteria. Such bacterial strains are disclosed in the application, e.g. in example 1, page 18. Next, the application discloses genes which can be used to modify said bacterial strains for efficient gene transfer, e.g. SEQ ID NO:1

and 2. The construction of plasmids and methods for transferring them into cells are disclosed e.g. in examples 3, page 28 and 4, 29. The release of the transferred DNA into the host cell is disclosed in detail in example 2, page 24, whereas suicide systems and the application of antibiotics may be used. Further, the application provides guidance to the skilled artisan how bacteria have to be cultured and how they are used as nutrients for animals, examples 5 and 6, page 30. Finally, in examples 7, page 31 and 8, page 32, it is explained to the skilled artisan which kind of somatic gene transfer can be achieved and how to monitor the success.

In summary, the disclosure of the pending application has to be regarded as sufficient to enable the skilled artisan to carry out the invention without any undue burden or undue experimentation. The skilled artisan, while using his general knowledge and the information provided by literature, together with the disclosure of the pending application was able to conduct *in vivo* gene transfer efficiently. Therefore, applicants believe that the application is in line with 35 U.S.C. §112, first paragraph.

Claim Rejections - 35 USC § 112 first paragraph; Claims 31-33

These claims stand rejected under 35 U.S.C. §112, first paragraph, as not fully comply with the requirements of the Budapest Treaty, 37 C.F.R. 1.801-1.809. Applicants hereby state that the organisms Listeria monocytogenes EGD Hyl_{D491A}, Listeria monocytogenes EGD Delta act A Delta PlcB and Listeria monocytogenes EGD Delta cspL 1 were deposited at the DSMZ (German collection of Microorganisms and Cell Cultures) under the terms of the Budapest Treaty, have been assigned accession numbers DSM

11881, DSM 11882 and DSM 11883, respectively, (patent application, English translation, page 20, last full paragraph, and paragraph bridging pages 20 and 21) and upon the issuance of a patent, all restrictions on the availability of theses accessions will be irrevocably removed. A copy of the Deposit Receipts for these deposits recognized under the Budapest Treaty is submitted herewith.

Accordingly, withdrawal of the rejection is respectfully requested.

For the reasons given above, Claims 23-34 are believed to be in condition of allowance, and a favorable action thereon and early issuance of the Notice of Allowance is earnestly solicited.

Respectfully submitted,

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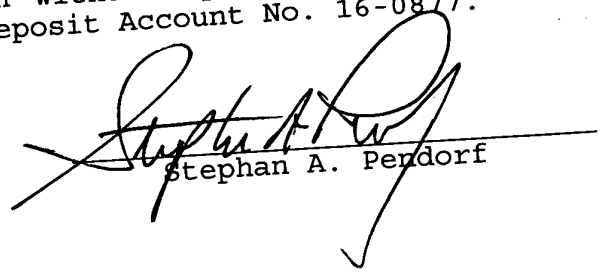
Date: July 3, 2002

CERTIFICATE OF MAILING AND AUTHORIZATION TO CHARGE

I hereby certify that the foregoing AMENDMENT A for U.S. Application No. 09/581,005 filed June 6, 2000, was deposited in first class U.S. mail, postage prepaid, addressed: Attn: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on July 3, 2002.

The Commissioner is hereby authorized to charge any additional fees, which may be required at any time during the

prosecution of this application without specific authorization, or credit any overpayment, to Deposit Account No. 16-0877.



Stephan A. Pendorf

VERSION WITH MARKINGS TO SHOW CHANGES MADE HEREBY ATTACHED

The Examiner is requested to accept the marked-up version as it is based on the previous version, which when modified as below, produces the clean version submitted with the current amendment.

IN THE CLAIMS:

Please amend the claims as follows:

31. A bacterial strain Listeria monocytogenes EGD Hyl[D]$_{D491A}$ which is deposited at the DSMZ (German Collection of Microorganisms and Cell Cultures) under the number of 11881 and is suitable for use according to claim 23.

ATTACHMENT

WORKING PROTOCOL

GENE TRANSFER OF PLASMID pERL3-CMV-GFP USING THE BACTERIAL STRAINS DISCLOSED IN THE PENDING APPLICATION (EGD Hly_{D491A} and EGD wildtyp)

DAY 1

- seed Hep2 cells ($1x10^5$ cells/well in a 24 well plate) in RPMI1640 medium without antibiotics, supplemented with 10 % FCS, 24 h, 37°C, 5 % CO_2
- prepare bacterial pre-cultures with EGD pERL3-CMV-GFP, EGD Hly_{D491A} , pERL3-CMV-GFP and as negative control, EGD without any plasmid
- prepare bacterial pre-cultures with *Listeria monocytogenes* strain EGD harboring plasmid pERL3-CMV-GFP, *Listeria monocytogenes* strain EGD HlyD491A containing pERL3-CMV-GFP and as a negative control *Listeria monocytogenes* strains EGD or EGD HlyD491A without any plasmid in 10 ml BHI medium, 12 h, 37°C, 200 rpm

DAY 2

- transfer bacteria 1:100 in 25 ml BHI medium, 37°C, 200 rpm
- culture bacteria till OD_{600} = 0.5 is reached, centrifuge, wash in RPMI1640 medium and chose appropriate number of cells

- wash Hep2 cells in RPMI1640 medium and add bacteria in a ratio of 50:1 to 5000:1 per cell

- incubate mixture containing bacteria and cells for 2 h, 37°C, 5 % CO_2

- discard bacteria, wash cells three times in RPMI1640 medium

- suspend cells in RPMI1640 medium with 20 % FCS and 50 µg/ml gentamycine to kill extracellular bacteria, 24 h, 37°C, 5 % CO_2

DAY 3

- discard medium from cells

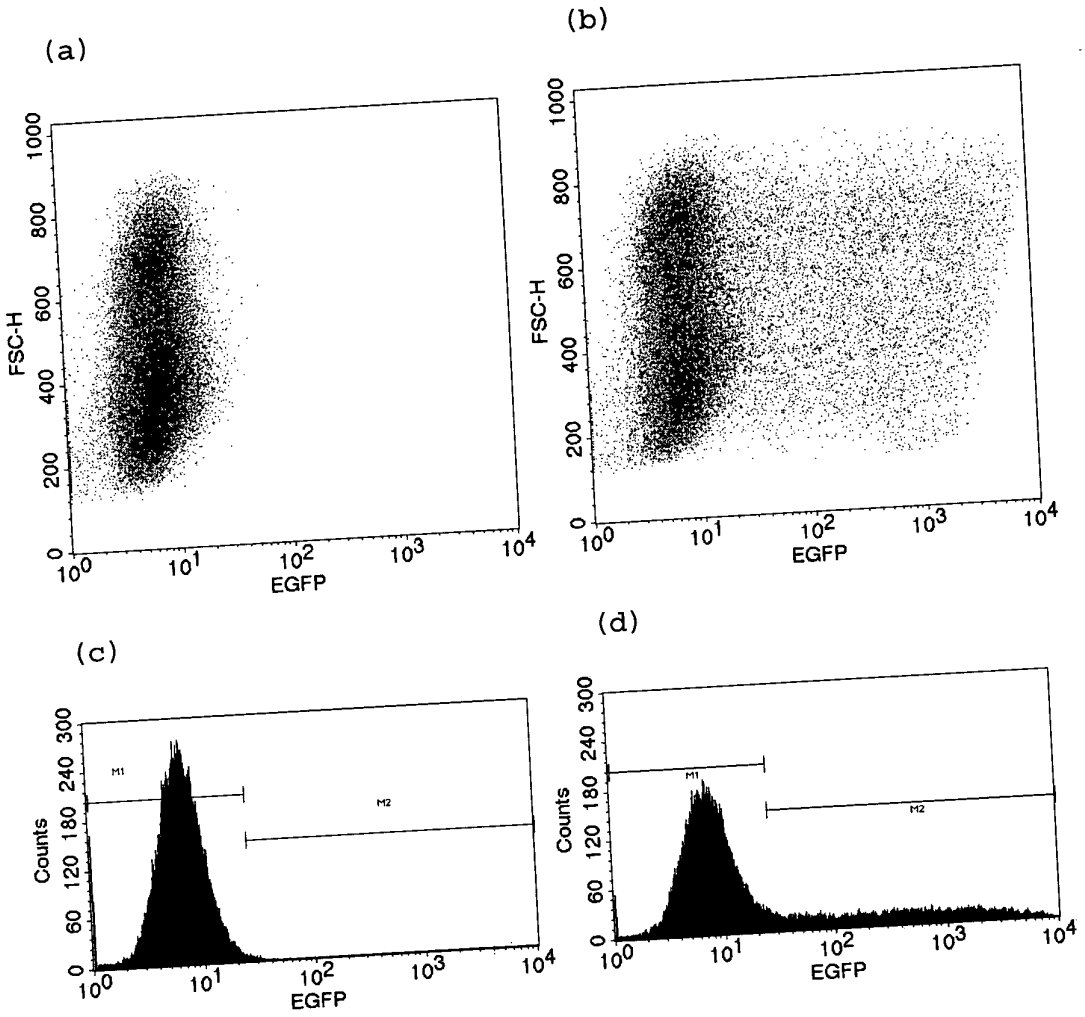
- replace it by RPMI1640 medium with 20 % FCS, 50 µg/ml penicilline/streptomycine to kill intracellular bacteria, 24 h, 37°C, 5 % CO_2

DAY 4

- FACS analysis of cells

For results see Fig. 1

Fig. 1:



(e)

EGD HlyD491A		pERL3-CMV-GFP EGD HlyD491A	
M1 [%]	M2 [%]	M1 [%]	M2 [%]
30,086 cells	69 cells	23,182 cells	6,640 cells
99,78	0,23	77,79	**22,28**

Efficiency of gene transfer using *Listeria monocytogenes* strain EGD Hly $_{D491A}$ is shown:

70,000 Hep2 cells were analyzed 48 h after transfection, the viability of the cells was confirmed with PI, Fig. 1 only shows PI negative, vital cell;

(a) and (c): Infection with EGD Hly $_{D491A}$

(b) and (d): Infection with EGD Hly $_{D491A}$ with plasmid pERl3-CMV-GFP, describe by Hense et al., 2001, Cell. Microb., 3:599-609

(a) and (b) show the fluorescence of EGFP (FL1-H) against FSC-H dot plots

(c) and (d) show EGFP (FL1-H) histograms

The statistic results of the histograms are shown in (e). It is pointed out, that up to **22.28 %** of the cells were efficiently transfected (marked also in bold in (e)).